Department of Biochemistry

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Research Activities

Cancer research

1. Glucose metabolism is another target for cancer chemoprevention. CD147 is an accessory subunit of heteromeric lactate transporters, monocarboxylate transporters (MCTs), known as the SLC16 family of solute transporters. The MCTs transport lactate across the plasma membrane, and the interaction of CD147 and MCT is required for expression of MCT activity and for the trafficking of MCT molecules to the plasma membrane. The pyruvate/lactate analog 3-bromopyruvate (3-BrPA) is a potent glycolytic inhibitor and candidate anticancer agent. Last year, 3-BrPA was shown, with the MCT1/small interfering RNA technique and a small molecular MCT1 inhibitor, to be transported into PC-3 cells through the CD147-MCT1 heteromeric lactate transporter complex and to promote cell death. The cytocidal activity of 3-BrPA against several cancer cell lines is enhanced under hypoxic conditions, because the expression of CD147 and MCT1 is greater than under normoxic conditions. To confirm the molecular chaperon function of CD147, the interacting proteins were screened with the co-immunoprecipitation method. Results revealed the existence of novel endogenous CD147-associated proteins, matrix metalloproteinase (MMP) 3 and carbonic anhydrases (CA9 and CA12), in addition to previously identified proteins, such as MMP1, MCT1, MCT4, and PDZ and LIM domain (PDLIM) 7.

2. Targeted chemotherapy against CD147-expressing carcinoma cells.
We studied the effects of anti-CD147 antibody-labeled polymeric micelles (aCD147ab-micelles) encapsulating a conjugate of glutathione and doxorubicin (GSH-DXR) on the specific accumulation and cytotoxicity against CD147-expressing human carcinoma cells. After treatment with an aCD147ab-micelle encapsulating GSH-DXR, a specific accumulation and cytotoxicity was observed in CD147-expressing cells.

3. E-cadherin suppression in epoxomicin-resistant cells may be regulated by expression of zinc finger E-box-binding homeobox (ZEB) 1. Six cell lines resistant to epoxomicin were established. The epoxomicin-resistant cell lines are reliable tools for evaluating the effects of proteasome inhibitors in preclinical trials. Moreover, these cell lines may also be useful for clarifying mechanisms of resistance to proteasome inhibitors and examining a wide variety of proteasomal functions. In an epoxomicin-resistant human endometrial carcinoma cell line, Ishikawa variant, E-cadherin gene (CDH1) expression was suppressed *via* overexpression of ZEB1, a transcriptional repressor of E-cadherin. Treatment of parental Ishikawa cells with epoxomicin immediately induced ZEB1, followed by transient suppression of E-cadherin expression. Expression of ZEB1 was followed by suppression of miR200 in epoxomicin-resistant endometrial carcinoma Ishikawa (Ish/EXM) cells, and expression of miR200 in Ish/EXM cells by means of

transfection of pre-miR200 repressed ZEB1 expression and recovered expression of E-cadherin. These results confirm that suppression of E-cadherin expression via ZEB1 expression is regulated by miR200 in Ish/EXM cells.

4. Targeting of the glycolytic pathway has become an attractive strategy for developing new anticancer agents. The pyruvate/lactate analog 3-BrPA is a potent glycolytic inhibitor. We have shown that 3-BrPA is transported into PC3 prostate carcinoma cells through MCT1 and promotes cell death. Then, we studied the cytotoxicity of 3-BrPA and protein expression of MCT1 using 20 different cell lines. The results suggested that 3-BrPA-sensitive cell lines tend to highly express MCT1 protein. Knockdown of MCT1 expression allowed several cell lines to survive despite treatment with 3-BrPA. On the other hand, MCT1 was expressed at low levels in the breast cancer cell line MDA-MB-231, which is resistant to 3-BrPA. We hypothesized that epigenetic silencing of MCT1 gene expression occurs in MDA-MB-231 cells. We found that a combination treatment of a DNA methyltransferase inhibitor (5-aza-2'deoxycytidine) and a corticosteroid (dexamethasone) significantly increased MCT1 mRNA expression in MDA-MB-231 cells. Thus, gene silencing of MCT1 in 3-BrPA-resistant cells is likely regulated through DNA methylation.

Other research

The following 3 studies were performed: 1) toxicity evaluation of chemicals by quantification of cellular polyubiquitin chains, 2) study of production of plasma proteins by well-differentiated human hepatoma cell lines, and 3) biochemical study of ubiquitin-specific peptidase 46 (USP46) underlying despair behavior in mice.

1. To find novel markers for assessing the risk of chemicals, we quantified cellular polyubiquitin chain levels in proximal tubular epithelial HK-2, neuroblastoma Neuro2A, and fibroblast NIH/3T3 cells exposed to $CdCl_2$, methyl mercury, or *N,N*,'-dimethyl-4,4'-bipyridinium dichloride (Paraquat). We found that Cd exposure induced a marked increase in polyubiquitin chains in all cells and, therefore, might be useful as a toxicity marker.

2. To develop plasma protein derivatives having no infectious risks, we tested human hepatocellular carcinoma FLC-4 and FLC-7 cells and found optimal culture conditions for the production of albumin and fibrinogen.

3. Deubiquitinating activity was measured in brain tissues of the CS mouse, which have lost despair behavior due to a gene mutation of USP46, and the activity was found to be reduced in the hippocampus and olfactory bulb of CS mice in comparison with that controls. To construct a measurement system for wild-type and mutant USP46 activity, we prepared an expression system using episomal vector in mammalian cells. The USP46 proteins (wild-type and mutant) were stably overexpressed in HeLa cells.

Publications

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